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植物遗传学

番茄GDP-D-甘露糖焦磷酸化酶cDNA的克隆、表达及定位

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摘要

GDP-D-甘露糖焦磷酸化酶催化GDP-D-甘露糖的合成，是植物抗坏血酸生物合成途径中上游的关键酶。以马铃薯GDP-D-甘露糖焦磷酸化酶cDNA序列为信息探针，在GenBank dbEST数据库中找到65条高度同源的番茄EST序列，通过序列拼接及RACE-PCR得到了番茄该基因的全长cDNA序列，命名为LeGMP。LeGMP 与马铃薯GDP-D-甘露糖焦磷酸化酶cDNA序列一致率为96%，推导的氨基酸序列与马铃薯、烟草、紫苜蓿、拟南芥的GDP-D-甘露糖焦磷酸化酶基因的一致率分别为99%、97%、91%、89%。经Northern杂交分析，LeGMP 在番茄根、茎、叶、花、果实中都有表达，但表达水平有差异。利用75个番茄远缘杂交重组系（IL系）将LeGMP 定位在番茄第3染色体上的D区段（3-D）。

关键词

番茄；GDP-D-甘露糖焦磷酸化酶；分子克隆；基因表达；定位

分类号

Cloning, Expression, and Mapping of GDP-D-mannose Pyrophosphorylase cDNA from Tomato (*Lycopersicon esculentum*)

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Abstract

<P>GDP-D-mannose pyrophosphorylase (GMP, EC 2.7.7.22) catalyzes the synthesis of GDP-D-mannose and represents the first committed step in plant ascorbic acid biosynthesis. Using potato GMP cDNA sequence as a querying probe, 65 highly homologous tomato ESTs were obtained from dbEST of GenBank and the putative cDNA sequence of tomato GMP was assembled. The full-length GMP cDNA of tomato was cloned by RACE-PCR with primers designed according to the assembled cDNA sequence. The full-length cDNA sequence contained a complete open reading frame (ORF) of 1 086 bp, which encoded 361 amino acid residues. This gene was designated as LeGMP (GenBank accession No. AY605668). Homology analysis of LeGMP showed a 96% identity with potato GMP and the deduced amino acid showed 99%, 97%, 91% and 89% homology with GMP from potato, tobacco, alfalfa and Arabidopsis thaliana, respectively. Northern blot analysis showed that LeGMP was constitutively expressed in roots, stems, leaves, flowers and fruits of tomato; but the expression levels varied. LeGMP was mapped to 3-D using 75 tomato introgression lines (ILs), each containing a single homozygous RFLP-defined chromosome segment from the green-fruited species Lycopersicon pennellii.</P>

Key words

tomato；GDP-D-mannose pyrophosphorylase；molecular cloning；gene expression；bin mapping

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